

The quest for an AIDS vaccine: is the CD8⁺ T-cell approach feasible?

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The rationale for developing anti-HIV vaccines that stimulate cytotoxic T-lymphocyte responses is given. We argue that such vaccines will work, provided that attention is paid to the development of memory T-cell responses that are strong and preferably activated. Furthermore, the vaccine should match the prevailing virus clade as closely as possible. Vaccines will have to stimulate a wide range of responses, but it is not clear how this can be achieved.

In sub-Saharan Africa, more than 25 million people are infected with HIV. The developing countries that are affected by this pandemic cannot afford the drugs to treat infected people. Even if drug prices were reduced, the costs that are associated with their clinical use are prohibitive. A prophylactic vaccine is urgently needed.

Initial efforts were aimed at producing an inactivated virus or recombinant envelope-protein vaccines^{1,2}, but it has been hard to stimulate the production of effective neutralizing antibodies. An envelope vaccine is currently in a phase III clinical trial in the United States and Thailand, and the first results are expected this year. However, the occurrence of several breakthrough infections in volunteers that were immunized with a similar glycoprotein (gp120) preparation in phase II trials³ has lowered expectations.

The initial steps that lead to HIV infection involve interaction of the envelope gp120 protein with CD4 and chemokine receptor molecules on the cell membrane. Therefore, gp120 is the primary target for HIV neutralization

with antibodies. Its structure explains why this is so difficult to achieve^{4,5}. Much of the outer surface of the protein is coated with carbohydrate and is not antigenic. The exposed polypeptide loops are highly variable and act as decoys for antibody; they are also easily altered by mutation. The conserved CD4 and CC-chemokine receptor 5 (CCR5)/CXCR4 binding sites are well hidden; the former is in a deep pocket and the latter is only exposed — from its guarding V3 loop — by a conformational change that occurs after CD4 binding. Three broadly crossreacting neutralizing sites that are recognized by human monoclonal antibodies

have been identified, but it has proven impossible so far to design candidate vaccines that can raise antibodies specific for them^{6–8}. Lack of progress has led to the exploration of other vaccine approaches.

The alternative approach is a vaccine that will elicit CD8⁺ T-cell responses, the potential of which has been shown by the success of attenuated simian immunodeficiency virus (SIV) in preventing SIV infection in rhesus macaques⁹. The main considerations are how to stimulate the right kind of CD8⁺ T cells effectively and how to deal with virus variability and its propensity to escape immune responses.

Natural history of CD8⁺ T cells

The study of anti-viral CD8⁺ T cells has been greatly enhanced by the development of new quantitative techniques, class I MHC tetramer staining¹⁰, the interferon- γ (IFN- γ) enzyme-linked immunospot (ELISPOT) assay¹¹ and analysis of intracellular cytokine production¹². In acute virus infections, there is a massive increase in the number of virus-specific T cells, which can be identified by tetramers. From a

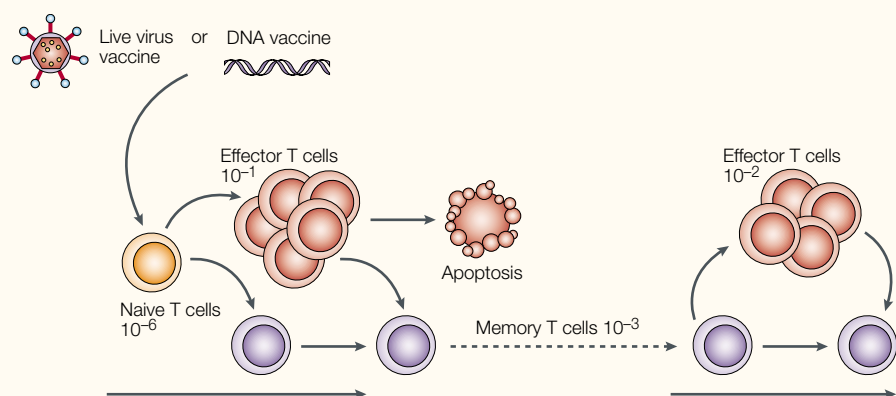


Figure 1 | **Generation of T-cell memory by vaccination.** Vaccination induces a strong CD8⁺ T-cell response in 14–21 days, by the stimulation of rare naive T cells. Antigen-specific T cells might expand to form 10% of all CD8⁺ T cells, but these expanded effector T cells die by apoptosis in the absence of persisting antigen. Memory T cells develop and are maintained at a frequency of <0.1%. They differ from naive T cells in being able to make interferon- γ in 6 hours. The horizontal arrows beneath the figure indicate when antigen or virus is present. Contact with virus stimulates a response from the CD8⁺ memory T cells. Although they make certain cytokines rapidly, they mature and divide to become effectors with potent lytic activity.

Table 1 | Examples of protection against virus infections mediated by CD8⁺ T cells

Virus	Vaccine	Challenge day after vaccine	Protection	Notes	Reference
RSV	DNA 50 µg i.m., M2 protein; day 0, day 21	2 days	Lung day 4: virus log ₁₀ 2.2 compared with log ₁₀ 3.6 in control animals	CTLs only; anti-IFN-γ blocks	121
RSV	Vaccinia M2, 10 ⁶ pfu i.p./i.n.; day 0	6 days 9 days 28 days	Complete ? Partial None		122
Influenza	DNA 100 µg i.m., NP; day 0, day 21, day 42	Not stated	100% survival compared with 0% of control animals	CTLs only	123
Influenza	DNA 100 µg i.m., NP; day 0, day 21, day 42	21 days	Lung day 7: 3 log ₁₀ reduction in virus load; 90% survival compared with 20% of control animals	CTLs only	79
LCMV	DNA 100 µg i.m., NP or NP-ubiq; day 0, day 14, day 28	42 days	Spleen day 4: NP, 2 log ₁₀ reduction; NP-ubiq, 5 log ₁₀ reduction		124
LCMV	Vaccinia-NP, 2×10 ⁴ pfu or 2×10 ⁶ pfu; day 0	7, 30 and 60 days	Low dose: survival at day 7, not day 30; high dose: survival to day 60		22

CTLs, cytotoxic T lymphocytes; IFN, interferon; i.m., intramuscular; i.n., intranasal; i.p., intraperitoneal; LCMV, lymphocytic choriomeningitis virus; M2, matrix protein 2; NP, nucleoprotein; pfu, plaque-forming units; RSV, respiratory syncytial virus; ubiq, ubiquitin.

precursor frequency of <1 in 10⁶ in an uninfected person, CD8⁺ T cells that react to a single immunodominant epitope can reach a frequency of >1 in 10 in about 20 days¹³. Most of these extra T cells die by apoptosis *in vivo* and the number of reactive T cells falls rapidly when antigen is cleared, which leaves a memory population of around 1 in 10³ peripheral blood mononuclear cells (PBMCs)¹⁴. If virus persists — for example, HIV, Epstein–Barr virus (EBV) and cytomegalovirus (CMV) — the number of T cells that are specific for dominant epitopes remains high^{15,16} and is probably maintained by continuous antigen-driven regeneration from memory cells. The differentiation status of these cells varies for different viruses^{17,18}, which is relevant to the different recombinant viral vectors under consideration as vaccines.

A virus-vectored vaccine that induces cytotoxic T lymphocytes (CTLs) would be expected to stimulate an acute response that is similar in magnitude to that found in an acute virus infection (FIG. 1). If the antigen did not persist, as in the case of replication-deficient viruses, this response would decay to leave memory T cells that would be activated only by later contact with HIV. This secondary response would not be able to neutralize the virus and could only abort the infection once the effectors were reactivated and in sufficient numbers. If vaccine antigen persisted, the activation state of the CTLs might be maintained and better suited to clear virus on later exposure.

Persistence of memory is a crucial issue for vaccines. Whether CD8⁺ T-cell memory depends on continued stimulation by antigen is controversial and seems to depend on the definition of ‘memory’ and the type of assay

used. Zinkernagel *et al.*¹⁹ showed that protection from viral challenge is dependent on repeated antigenic stimulation of CD8⁺ memory T cells to differentiate into effector CTLs. Immunization with short-lived antigens gives good protection against challenge, but only for a very short period; as the antigen disappears, the protective effect vanishes (TABLE 1). By contrast, Ahmed and colleagues²⁰ measured re-induction of the CD8⁺ T-cell response *ex vivo* and showed that memory cells persist for a very long period in the absence of antigen. Cytokines such as IFN-α are necessary to maintain this form of memory in the absence of antigen²¹. Zinkernagel *et al.*²² were probably measuring activated CD8⁺ effector T cells, which are able to kill and produce a range of cytokines, whereas Ahmed and others²⁰ measured the resting long-lived memory-cell population, which is generated after the initial burst of effector-cell production. The crucial question for vaccine development is whether it is necessary to maintain fully active effector cells or whether the antigen-independent memory — which can be reactivated rapidly by antigen — is sufficient. If fully mature effectors are needed, is it necessary to design vaccines that persist? However, this is currently anathema to the regulatory bodies because it would be extremely hard to guarantee long-term safety.

The role of CD4⁺ T-cell help in determining the state of CD8⁺ T-cell memory is poorly understood^{23,24}. In the absence of CD4⁺ T-cell help, CD8⁺ T cells were shown to be dysfunctional²⁵, an observation that is consistent with some findings in HIV-infected patients¹⁷, in whom CD4⁺ T-cell help is impaired. Priming of CD8⁺ T-cell responses might require linked epitope recognition by CD4⁺ T cells²⁶. The

initial induction of CD8⁺ T-cell responses requires interleukin-12 (IL-12) production²⁷ by ‘LICENSING’ of dendritic cells by CD4⁺ T cells^{28–31}, but some viruses can bypass this requirement. CD4⁺ T-cell help can also maintain CD8⁺ T-cell memory^{32,33}. CD4⁺ T cells have direct antiviral effects — for example, IFN-γ and β-chemokine production³⁴ — but CD4⁺ T cells are less important than CD8⁺ T cells in this regard; they can only attack infected cells that express class II MHC proteins. The early loss of HIV-specific CD4⁺ T cells in HIV infection and the subsequent progressive loss of all CD4⁺ T cells probably undermines the CD8⁺ T-cell response to HIV³⁵.

Vaccines that stimulate CD8⁺ T cells are also likely to stimulate CD4⁺ T helper (T_H) 1 cells. This could be one advantage that a vaccine has over natural HIV infection. Indeed, good early CD4⁺ T-cell responses to HIV are associated with lower virus loads and better prognosis^{24,36}. The possibility of inducing an immune response that is more effective than the natural response to HIV infection addresses the concern that there is no unequivocal case of an infected person clearing the infection; this would be an unprecedented premise on which to base the development of a vaccine.

Assays for virus-specific CD8⁺ T cells

Measurement of CD8⁺ T-cell responses has been revolutionized by the introduction of tetramer-¹⁰ and cytokine-staining techniques. Tetramers detect antigen-specific T cells by their ability to bind tetrameric human leukocyte antigen (HLA) class I molecules folded around a particular peptide, but this does not indicate function. The cytokine assays measure antigen-stimulated cytokine production

(typically IFN- γ), which is either captured on an antibody-coated surface¹¹ or retained within the cell by brefeldin-A treatment, which blocks exocytosis. In the latter case, measurement is completed with intracellular staining by fluorescent antibody specific for cytokine and flow cytometry¹². These assays measure T-cell function but they might underestimate the specific T-cell numbers. How these assays compare with each other and with the fresh PBMC cytotoxicity assay³⁷ and limiting-dilution assay^{16,38} is shown in TABLE 2. It is clear that the tetramer and cytokine-release assays are the most sensitive and accurate.

CTLs and non-HIV infections

Because CTLs cannot neutralize virus, it is important to know whether a CTL response alone can protect against virus infections. CTLs would have to act by killing infected cells and aborting an infection, rather than by preventing it. The state of the CTLs might be crucial: do they have to be active effectors or can memory cells do the job? Memory cells can secrete cytokines within six hours of antigen contact¹¹, but maturation to the killer phenotype takes longer.

Important studies in mice have shown that CTLs that are induced by vaccination can protect against viral disease (TABLE 1). The protection was never sterilizing, in that mice were still infected but with less virus, and the beneficial effect was usually measured by survival after challenge with a lethal dose of virus. Virus was usually detected after challenge, but at much lower titres in the vaccinated animals compared with controls. Control of the infection by the vaccine-induced memory T-cell response was probably enhanced by the immune response that was triggered by the actual infection. When the challenge was within 14 days of vaccination, the T cells were likely to be recently stimulated effectors (FIG. 1); in later challenges, protection would have to be mediated by long-term memory cells. Protection tended to be better soon after vaccination and was often poor at later times, although this was not always the case in the macaque vaccine and challenge studies (see below); this issue needs further rigorous investigation.

CTLs in the control of HIV infection

The CD8⁺ T-cell or CTL response is crucial in controlling HIV and SIV infections over a period of several years, although ultimately the control breaks down. The appearance of CTLs corresponds to the time when the initial viraemia comes under control and starts to fall^{39–41}. Thereafter, there might be an inverse relationship between virus load and

Table 2 | Comparison between CD8⁺ T-cell detection assays

Assay	Equivalence		Sensitivity
	Virus-specific (% CD8 ⁺ T cells)	Calculated specific CD8 ⁺ T cells per 10 ⁶ PBMCs	Lower limit per 10 ⁶ PBMCs
Tetramer stain	1.0%	3,000	100
IFN- γ ELISPOT	0.3%	1,000	50
ICA	0.3–1.0%	1,000–3,000	100
Direct lysis at a ratio of 50:1	5–10% specific lysis	NA	5–10% lysis
LDA	0.1%	300	10

The table shows the assays that are available to measure CD8⁺ T cells. For the direct lysis assay, fresh uncultured PBMCs are added to virus-infected or peptide-pulsed target cells. The equivalence values shown in the middle two columns indicate the values that would be expected for a typical HIV-positive blood sample, tested in the different assays. The calculated specific T cells are the values indicated by the different assays; note that most assays underestimate compared with direct tetramer staining. The right-hand column shows the lowest values that are detectable in each assay. Note that the sensitivity of the LDA is offset by its tendency to underestimate the actual number of antigen-specific T cells by a factor of 10–100 (REF. 16). ELISPOT, enzyme-linked immunospot; ICA, intracellular cytokine assay; IFN- γ , interferon- γ ; LDA, limiting-dilution assay; NA, not applicable; PBMCs, peripheral blood mononuclear cells.

the number of specific CTLs¹⁵ (but, see REFS 42,43). Better evidence comes from macaques, for which the infusion of anti-CD8 monoclonal antibody *in vivo* abolished the control of viraemia^{44–47}. When the antibody was infused in the acute phase of infection, the initial high viraemia was not brought under control until the antibody-mediated reduction in CD8⁺ T cells had faded. Similarly, when anti-CD8 antibody was infused during chronic infection, the virus level immediately rose, only to fall when CD8⁺ T cells returned.

The effectiveness of CD8⁺ T cells in controlling HIV infection is shown by the selection of virus escape mutants^{48–52}. When virus is abundant, with high turnover (particularly in early or late infection), escape mutants are selected. Escape can occur by mutation at more than one epitope site in the same time period⁵². Such strong selective pressure is indicative of the very potent antiviral effect of CTLs.

The efficiency of virus control that is achieved by the CD8⁺ T cells is undermined by virus escape and variability. This is probably made worse by the downregulation of expression of HLA class I molecules that is mediated by Nef^{53,54}. Progressive impaired function of CD8⁺ T cells must also contribute to poor virus control as the CD4⁺ T-cell number falls and their function becomes negligible.

Are HIV-specific CTLs protective?

In all cohorts of people who are exposed to HIV, about 5% of individuals seem to be resistant to HIV infection. In a Nairobi sex-worker cohort⁵⁵, women were very highly exposed, with several HIV contacts per year. Their T cells were fully infectable *in vitro* and did not express defective virus receptor genes such as CCR5 Δ 32, a mutant that prevents the surface expression of CCR5 and almost completely

prevents virus entry into the cell. The T cells did not induce anti-HIV immunoglobulin G⁵⁶ but made CTL responses to HIV⁵⁷. Some of the women became infected with HIV after ceasing sex work, which implies that they need repeated antigenic stimulation by HIV of CTLs to maintain protection.

Similar resistance to HIV has been found in uninfected babies whose mothers are HIV-positive⁵⁸, in medical staff exposed to HIV by needle-stick injury^{59,60} and in long-term uninfected partners of HIV-positive individuals⁶¹. Macaques that were treated with antiretroviral drugs within hours of SIV infection controlled the infection with undetectable levels of virus, were protected against re-challenge and made SIV-specific CTLs^{62,63}.

Vaccines that induce CD8⁺ T-cell responses can protect monkeys from challenge with SIV or even HIV (TABLE 3). The DNA and recombinant virus vaccines that protect against the highly aggressive simian-human immunodeficiency virus SHIV-89.6P are particularly impressive^{63–66}. After challenge, these monkeys were infected but the infection was greatly attenuated, so that the virus load was lower than in controls by a factor of 1,000 and the CD4⁺ T-cell count remained normal for more than a year after challenge. Therefore, the vaccinated animals survived without sickness compared with controls, most of which experienced rapid loss of all CD4⁺ T cells and early death. It is pertinent that one protected animal succumbed to AIDS more than a year after challenge — the virus had mutated the dominant epitope that was recognized by the vaccine-induced CTLs⁶⁷. This strongly supports an important role for CTLs in the protection of these animals, but it also gives a warning of possible problems for a human CTL-inducing vaccine.

Table 3 | Protection of macaques from SIV or SHIV challenge by CD8⁺ T-cell-inducing vaccines

Vaccine	Challenge virus	Result	Notes	Reference
Vaccinia–Nef	SIVmac J5	Reduction of virus load		105
MVA–Gag+Pol+Env	SIV SHIV-89.6P	50-fold reduction of virus load 100-fold reduction of virus load		125 103
DNA+fowlpox–Gag+Pol+Env	HIV-1 6 weeks later	Protection but not sterilizing immunity	Infection of <i>Macaca nemestrina</i>	99
DNA+MVA–epitope	SIVmac 1 week later SIVmac 1 week later	Partial protection? <1 log ₁₀ virus reduction	CTLs specific for only one epitope	68; J. M. Allen, personal communication
DNA+IL-2–Gag+Env	SHIV-89.6P 6 weeks later	Survival, no loss of CD4 ⁺ T cells 3 log ₁₀ reduction of virus load		63
DNA+MVA–Gag+Pol+Env	SHIV-89.6P 7 months later	Survival, no loss of CD4 ⁺ T cells, 3 log ₁₀ reduction of virus load	DNA prime included other HIV genes; mucosal challenge	64
VSV–Env+Gag	SHIV-89.6P 3–6 months later	Survival, no loss of CD4 ⁺ T cells, 3 log ₁₀ reduction of virus load	Early control probably CD8 ⁺ T-cell-mediated	65
Adenovirus–Gag	SHIV-89.6P	Survival, no loss of CD4 ⁺ T cells, 3 log ₁₀ reduction of virus load		66
Peptide+adjuvant	SHIV-Ku2	Reduced virus load, no CD4 ⁺ T-cell loss	Mucosal immunization and challenge	126

CTLs, cytotoxic T lymphocytes; env, envelope protein; gag, group-specific antigen; IL, interleukin; MVA, modified vaccinia Ankara; pol, polymerase; SHIV, simian–human immunodeficiency virus; SIV, simian immunodeficiency virus; SIVmac, macrophage-tropic SIV; VSV, vesicular stomatitis virus.

These results are remarkably similar to those obtained in mice that were vaccinated to stimulate CD8⁺ T-cell responses and then challenged with high doses of virus (TABLE 3). However, it might, paradoxically, be more easy to protect against the aggressive SHIV-89.6P than against a more insidious virus. Similarly, strong CTL responses were poor at controlling an unmodified SIV that is less aggressive⁶⁸ (D. Watkins, unpublished observations). The reasons for these differences need to be resolved.

A vaccine that does not stop infection but reduces virus load might not be ideal, although it should improve prognosis in infected vaccine recipients⁶⁹. The dose of virus challenge used in the macaque experiments is very high, and was chosen to infect all of the control animals reliably. This dose is probably more than 100 times the dose that is associated with human sexual contact, so it might be easier to protect people who are naturally exposed to HIV at low doses, albeit repeatedly. This type of challenge virus exposure should be tested in vaccinated macaques.

These data form the basis for several vaccine studies in humans. They all test the same hypothesis — that a strong CD8⁺ T-cell response will protect against HIV infection.

Stimulation of CD8⁺ T cells by vaccines

It has long been known that many classical vaccines — for example, inactivated influenza virus vaccines and haemagglutinin vaccines — are poor at stimulating virus-specific CD8⁺ T cells^{70,71}. Conversely, a live attenuated measles vaccine elicited a strong CD8⁺ T-cell

response in humans⁷². Many recombinant attenuated virus vectors (for example, vaccinia, adenovirus, influenza and Semliki Forest virus) and recombinant intracellular bacterial vaccines (for example, *Mycobacterium bovis* bacillus Calmètte–Guerin (BCG)) have stimulated CTLs in mice. Inert virus-like particles, which are taken up by dendritic cells, have stimulated good CTL responses in mice, although not in primates and humans^{73–75}. Peptides can also be effective, although the response might be short lived⁷⁶. So, any vaccine that can enter the class I MHC antigen-processing pathway works well (FIG. 2). However, the more complex recombinant virus vaccines might stimulate a response to irrelevant antigens, such as some of the 200 vaccinia proteins that might also be expressed by infected cells alongside the insert. As CTL responses tend to be focused on few epitopes — sometimes only one^{77,78} — this can mean that a good response is achieved, but not to the insert. This problem can be avoided by using a DNA vaccine that comprises only the desired insert (possibly with an antibiotic resistance gene). Plasmid DNA stimulates a well-focused response in mice after only one injection (intramuscular, intradermal, intravenous, intranasal, intrarectal, intravaginal or intraperitoneal)^{68,79–81}.

CTL induction in primates

There is now a great deal of effort focused on plasmid DNA and recombinant virus vaccines. There has been uncertainty over whether DNA vaccines would work in humans, but early indications are that DNA

is safe and stimulates weak CD8⁺ T-cell responses in some volunteers^{82,83}. It makes sense that HIV proteins that are expressed early in the HIV replication cycle, such as Nef, Rev, Tat and Env, should be important immunogens for vaccine design. So, an anti-HIV DNA vaccine that expresses Rev and Env was given to HIV-infected and uninfected people; it stimulated non-neutralizing antibody, antigen-specific T-cell proliferative responses and production of macrophage inflammatory protein-1 α (MIP-1 α), but no CTLs^{84–87}. CTLs were stimulated in asymptomatic HIV-infected patients by DNA that encodes HIV Tat, Rev and Nef^{88,89}. CTL responses were weak, but most of these studies used suboptimal methods for detecting antigen-specific CD8⁺ T cells. At least one phase I trial of DNA that encodes HIV antigen is in progress in HIV-negative volunteers.

Recombinant vaccinia viruses have been used extensively in inbred mice to stimulate CTLs^{90,91}. Recombinant vaccinia viruses and related poxviruses that were given to primates and humans in phase I trials stimulated variable and, in humans, generally weak CD8⁺ T-cell responses^{92–96}. Because of safety concerns with vaccinia virus, attenuated or related viruses have been proposed as vectors: canarypox^{92,93,95,97,98}, fowlpox⁹⁹, NYVAC (New York vaccinia virus with 18 gene deletions selected to decrease pathogenicity)^{98,100} and modified vaccinia virus Ankara (MVA)⁶⁴. These all infect human cells, but the virus replicates very poorly or not at all. MVA was passaged more than 500 times in chick-embryo fibroblasts so that it accumulated large deletions and was no

longer able to replicate in human cells, although they were infected^{101,102}. It was given to more than 100,000 people as a smallpox vaccine, with no reported side effects^{101,102}. These attenuated and avipox viruses, which are recombinant for HIV proteins, show promise in macaques^{98,99,103,104}. Recombinant MVA has entered trials for both HIV and malaria. In the former, some strong (>500 ELISPOT spot-forming units per million PBMCs) responses were seen, but not in all vaccine recipients (M. Mwau *et al.*, unpublished observations).

An inconsistency in the use of recombinant poxviruses is that not all recipients in outbred species respond. Not all macaques make a CD8⁺ T-cell response (for example, REF. 105) unless the response is deliberately focused on known immunodominant epitopes. This is typical of immunodominance, whereby the CTL response, which is determined by MHC type, focuses on few epitopes and might not respond to the inserted sequence despite a good response to the whole virus.

The relative inefficiency of single-vaccine modalities in humans and primates has led to methods of augmenting vaccine immunogenicity. Barouch *et al.*⁶³ reported good responses with 5 mg DNA plus IL-2-Fc fusion protein (IL-2 fused to the Fc fragment of immunoglobulin to greatly increase its half-life *in vivo*) or DNA that encodes IL-2-Fc. Immunization with DNA-coated microparticles can target the dermal-epidermal junction region of the skin, which is rich in Langerhans cells, and this has stimulated CTL responses at low DNA doses^{106,107}. When mice were primed with plasmid DNA and then boosted with MVA recombinant for the same DNA sequence, a CTL response tenfold greater than for either vaccine alone was observed^{80,81}. The DNA might prime a focused response that is then amplified by the virus boost. This approach works well in macaques^{64,68,99,107} (TABLE 3) and is now in phase II trials in humans.

What is a good CTL response?

Very little attention has been focused on what constitutes a good CTL response. There are few data on the level of CTL response that is needed to protect. Sex workers who are exposed but uninfected are protected from HIV infection and make CD8⁺ T-cell ELISPOT responses of around 50–100 spot-forming units per 10⁶ PBMCs — a level that should be easily achievable with a vaccine^{57,108,109}. However, they need continuing exposure to virus to maintain their protection, so for a non-persisting vaccine, higher levels of CTLs are likely to be needed¹⁰⁹. Also, it is not clear how broad the response in the

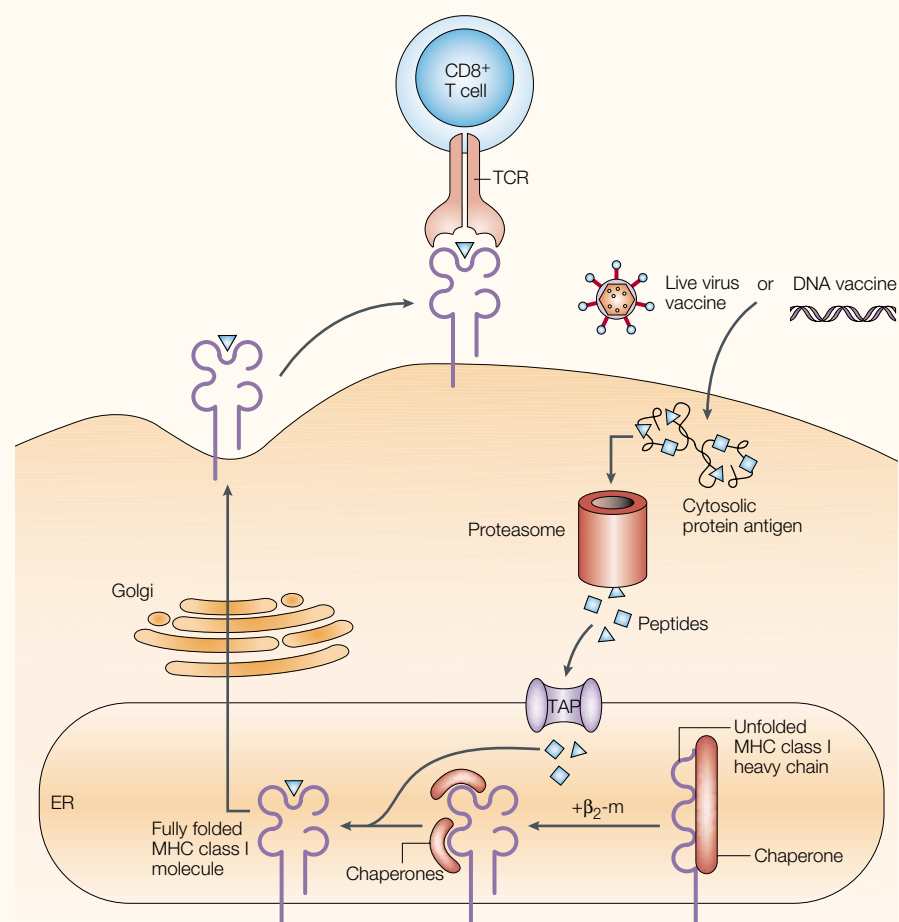


Figure 2 | **MHC class I processing and presentation of antigens.** CD8⁺ T-cell vaccines enter the class I antigen processing pathway to generate HLA-class-I-peptide complexes on the surface of antigen-presenting cells. Cytosolic protein antigens are generated as a result of vaccination. These are degraded into peptides by the proteasome and transported into the ER. In the ER, peptides become associated with newly generated MHC class I molecules and are transported to the cell surface where they stimulate CD8⁺ T cells. β_2 -m, β_2 -microglobulin; HLA, human leukocyte antigen; ER, endoplasmic reticulum; Tap, transporter for antigen processing; TCR, T-cell receptor.

sex workers is, and their total response might be two or three times higher than susceptible individuals; it is intriguing that they seem to respond to different epitopes than infected people, which indicates that not all epitopes are equal in this regard⁵⁷.

Macaques that were immunized using a PRIME-BOOST protocol with DNA and MVA Gag, which induced a very large CD8⁺ T-cell response to a single epitope (up to 5% of all CD8⁺ T cells specific for p11C, C-M presented by Mamu A*01) were not protected against a challenge with a high dose of moderately aggressive SIV⁶⁸ (D. Watkins also has similar data with the same epitope vaccine; personal communication). So, a response to more than one epitope might be needed and, again, choice of epitope could be crucial. The animals that were protected against SHIV-89.6P challenge generally had peak specific CD8⁺

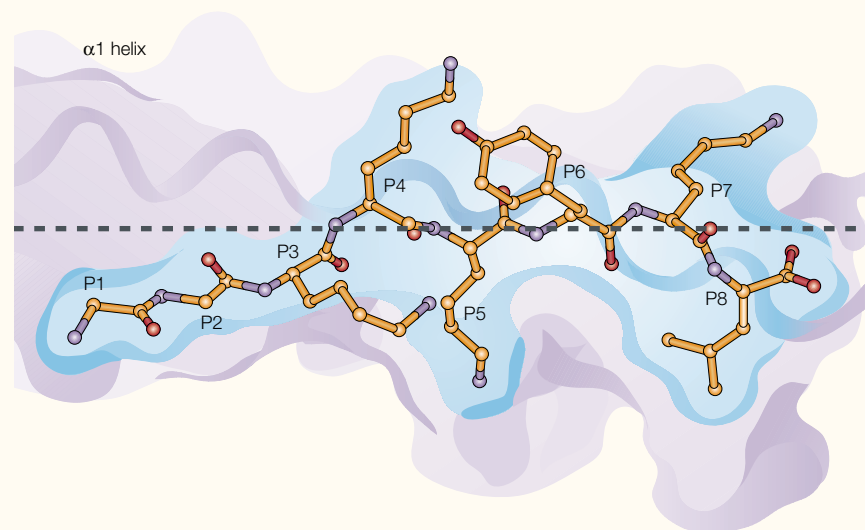
T-cell levels between 1% and 10% for known epitopes — by tetramer staining — and probably further responses to other epitopes^{63,64,103}, but these responses did not persist at this level before challenge.

The studies on virus challenge might show what level of response is needed for each macaque model, but these experiments do not mimic repeated low HIV dose mucosal exposure with a range of variant viruses. In the absence of relevant information, we can only make guesses on the basis of the above models and what we know about control of HIV infection in chronically infected humans. We propose that peak responses in excess of 300 IFN- γ ELISPOTS per million PBMCs to more than one epitope should be achievable and comparative to the macaque data. It is a concern that trials of vaccines that stimulate only a weak CD8⁺ T-cell response might fail.

Box 1 | Clades matter

HIV clades differ by 7–15% in their amino-acid sequences and within the clades, there is significant variation¹¹⁹. The length of a cytotoxic T lymphocyte (CTL) epitope is eight to ten amino acids. So, on average, each epitope will differ by one amino acid between clades. Although there are claims for clustering of epitopes in conserved regions of HIV proteins, there has been a bias in ascertainment in identifying conserved epitopes, because the reagents used are based on consensus amino-acid sequences and might not match the infecting virus. Because HIV can escape from CTLs by mutation, and then be transmitted¹²⁰, epitopes could even cluster in the more variable parts of the virus. Here, it is reasonable to assume a roughly even distribution of variability in epitopes; that is, one amino acid per epitope.

The figure illustrates how a peptide binds to a human leukocyte antigen (HLA) class I molecule. In this typical example, HIV Gag p17 24–31 GGKKKYKL is situated in the groove of HLA-B8 (REF. 114). Three amino-acid side chains at P4, P6 and P7 point out towards the T-cell receptor (TCR) (above the dashed line), three — P3, P5 and P8 — bind into the groove and two — P1 and P2 — are neutral. Mutations of the TCR-interacting or the HLA-binding amino acids affect T-cell recognition¹¹⁴. Burrows *et al.*¹¹² thoroughly examined the effect of mutation for another peptide in Epstein–Barr virus EBNA3A-specific CTLs; he made all 171 single amino-acid changes in the nonamer peptide; those in the six HLA-binding and TCR-interaction positions severely damaged T-cell recognition by the T cells. This pattern is typical for HLA-B8, but the principles are the same for peptides that bind to other HLA class I molecules. Therefore, it should be expected that two thirds of epitope mutants affect T-cell recognition and that CD8⁺ T cells will cross react poorly across clades. Reproduced, with permission, from REF. 114 © (1996) The Rockefeller University Press.



Another unknown quantity is the T_H1 CD4⁺ T-cell response that will accompany a vaccine-induced CD8⁺ T-cell response. This is likely to be better than that induced by natural HIV infection, during which CD4⁺ T cells are preferentially infected by HIV and destroyed^{36,110}, and so might work in the vaccine's favour. In general, T_H1 responses to acute virus infection are present but smaller than the CD8⁺ T-cell responses¹². A good vaccine would stimulate this type of response, which might be useful for the vaccine recipient who becomes infected with HIV. The rare non-progressors or very slow progressors after HIV infection have good T_H1 responses¹¹⁰. Therefore, any T_H1 response that is stimulated by the vaccine is likely to be beneficial. This could be offset by the particular susceptibility of these T cells to HIV infection, but this is generally discounted.

Clades

The design of an HIV vaccine is complicated by the virus variability in amino-acid sequence. Most studies in macaques have not addressed this issue, and have matched challenge virus to the vaccine. There is some confusion as to the importance of CLADE differences in the CTL response. The data that are available on cross-clade recognition by T cells are limited to a small number of T-cell clones and lines, which are specific for a tiny fraction (<1%) of the total repertoire of HIV epitopes that are seen by human CTLs. Therefore, reports of good cross-reaction or non-cross-reaction can not be representative. Until vaccine recipients can be thoroughly tested for cross-clade reactivity in their T-cell responses over a range of peptide concentrations, a more theoretical approach is appropriate. As argued

in BOX 1, two thirds of point mutations in an epitope are likely to adversely affect T-cell recognition. If epitopes are more or less evenly distributed across virus proteins, each will have one or more changes when two clades are compared. There have been theoretical arguments for epitope clustering¹¹¹, but experimentally the question is still open. If most epitopes differ between clades, this would have serious implications for vaccine design.

As shown in BOX 1, mutations can interfere with peptide binding to the human leukocyte antigen (HLA) molecule. Such binding is very sensitive to change, and mutation removes the epitope. Changes that affect T-cell recognition are more subtle. Depending on the affinity of the interaction, there might be no effect (which is rare)¹¹², reduced affinity leading to T-cell antagonism and impaired responses^{113,114}, or no recognition. In the last case, we might expect a new response that can recognize the peptide to take over, but this often does not happen — a concept that is referred to as 'original antigenic sin'¹¹⁵.

Therefore, a vaccine that stimulates a response to a single epitope would be very vulnerable to variation in the virus sequence. It would give only poor protection against a different clade of HIV — 33% of that seen with homologous virus exposure, and even virus with the same sequence as that recognized by the induced CTLs would be susceptible to escape by mutation. The latter would be a significant problem if the infection was not controlled before much virus replication occurred. If the vaccine could generate a response to several epitopes the problem would be lessened: a five-epitope response would have an 87% chance of cross-clade recognition of at least one epitope. However, in such a five-epitope response, a third of the responders would recognize only a single cross-reactive epitope and would therefore be very susceptible to virus escape. This is a real problem that has to be addressed in vaccine design.

Virus escape

Escape of SIV mutants after vaccination has already been reported^{67,116}. In macaques that were protected against SHIV-89.6P infection by a vaccine, such escape was associated with rapid progression to AIDS⁶⁷. In acute SIV infection, escape mutations at several epitopes are selected by the CTL response⁵². Escape from CTLs also occurs in acute HIV infection^{51,117,118} and must be important in the failure of immune control.

Such escape could seriously undermine vaccine prophylaxis of HIV. The only defence is to induce responses to several epitopes at the same time. It is encouraging that

triple-drug therapy does not seem to select significant escape mutation, unlike single- or double-drug therapy; part of the protection must be due to the drug-induced low level of virus replication. This gives some hope that if the immune response is activated very early in infection, there is a chance that the virus could be controlled by a response to at least three epitopes before high-level virus replication occurs. Given the above arguments about pre-existing virus variability, this could mean inducing CTL responses to ten or more epitopes.

Breadth of the immune response

A remarkable feature of the natural T-cell response to acute or chronic virus infection is that the CD8⁺ T-cell response can be focused on a very small number of epitopes^{77,78}. In the CD8⁺ T-cell response to acute EBV infection, as many as 40% of blood CD8⁺ T cells can respond to a single epitope¹³, despite the fact that this herpes virus expresses hundreds of proteins. This type of CTL response could be disastrous for a vaccine, as it offers an easy escape route. It is not clear how to broaden a vaccine response, and the obvious possibility of adding more virus proteins to the vaccine might not work (as for EBV). It might be better to mix several small vaccine constructs together, fooling the immune system into responding to several 'invaders', each requiring a strong T-cell response. For a DNA prime and recombinant virus boost schedule, it might only be necessary to do this for the DNA priming component.

Duration of the immune response

In macaques that were immunized with non-replicating MVA, the half life of tetramer-stained CD8⁺ T cells seems to be around seven days⁶⁸. The memory T-cell response that remains is at a much lower level. This is probably typical of the response to a non-persisting antigen. If a high level of mature effectors is required for protection, continuous or repeated antigenic stimulation will be required. The evidence from the Nairobi sex workers indicates that this will be needed, at least for complete protection; in several cases, susceptibility to HIV infection was restored when they ceased prostitution¹⁰⁹. However, their concentration of antigen-specific CD8⁺ T cells while they were protected was less than that which can be induced in humans by a vaccine, so the situation might not be exactly comparable. Amara *et al.*⁶⁴ found that their macaques were protected against SHIV-89.6P disease when challenged seven months after the last immunization. By contrast, there was little or no protection in macaques challenged

at the peak of the tetramer response to a single epitope⁶⁸ (T. M. Allen, T. Hanke and D. Watkins, unpublished observations).

The SHIV-89.6P-challenge studies indicate that useful but incomplete protection can be obtained by long-lasting memory T cells; complete protection might need higher levels of fully activated effectors. Only phase III efficacy trials will show whether CTL memory that is induced by current vaccines will work. If non-persisting vaccines do not protect, persisting antigen vaccines will have to be tested. The regulatory authorities will have to confront this need.

Why the idea might be right but fail

The animal studies that have been discussed show that the CTL-vaccine approach can work. However, for HIV, conditions will have to be exactly right. There is a danger that one or two negative trials could kill the whole idea of a CTL vaccine. Therefore, it is vital that conditions for the first efficacy trials are optimal. The reasons that a vaccine might fail have been discussed: the vaccine-induced T cells might have to be in an activated state that cannot be maintained by the vaccine, the immune response might be too weak, the T cells might not see enough epitopes to cope with virus variability, the virus might escape from the T-cell response or the duration of protection might only be brief. Even at best, a CTL-inducing vaccine might be only half a vaccine — that is, it might only really protect in combination with neutralizing antibody.

These concerns combine to produce a formidable challenge, but one that cannot be avoided. There is now a CTL-vaccine bandwagon, with several teams gearing up to test the same hypothesis. One bad trial could ruin

the whole lot, although a good trial that gives a clear negative answer would be scientifically very important. A positive protective effect would open the door to a vaccine and could be within our grasp soon.

Trials: ethical and political issues

The need for an HIV vaccine is desperate in developing countries. Apart from a few exceptional sites, only these countries have a high enough incidence of HIV infection to conduct phase III efficacy trials. Therefore, it is essential to establish strong collaborations well in advance of such trials. Matching of clades between vaccine and the most prevalent virus has been used as a political argument to ensure that such collaborations are truly in the interests of the African or Asian partner. In fact, there are stronger scientific arguments as to why the clades should be matched in any phase III efficacy trial. It is also important to have an outline plan for further vaccine development to ensure that, if the vaccine works, it will be made available in the partner country at the earliest opportunity. The medical, scientific and regulatory authorities are well aware of these issues, and trials need at least two years of preparation to deal with these issues before the trial itself. The associated infrastructure development needs similar forward planning.

Thought also has to be given to serious ethical issues. The trials will only give answers if some control volunteers become infected with HIV. If the vaccine only partly protects, or does not work, vaccine recipients will also be infected. The level of treatment that they should be offered — for life — needs very careful discussion, which must involve the community to which the trial participant belongs. For these and more commercial reasons, vaccines that are targeted at developing countries are not attractive to the pharmaceutical industry. Alternative funding streams have been created and need continued support (for further discussion of these issues, see <http://www.iavi.org>).

Conclusions

HIV presents an unprecedented challenge to vaccine design and conduct of trials. Virus variability is a particularly serious problem. It must not be assumed that 90% similarity between HIV clades means that a vaccine that is based on one clade will give 90% protection against another clade; it is more likely that such cross protection would be as little as 33%. Efforts must be made, therefore, to ensure that the vaccine stimulates a broad response. This is difficult to ensure, given the tendency of the immune response to focus on only a few

Glossary

CLADE

A subgroup of HIV variants with a greater degree of genome homology.

LICENSING

The activation of dendritic cells by CD4⁺ T cells through CD40–CD40L interaction.

PRIME-BOOST

When a single application of a vaccine is insufficient, repeated immunizations are performed using the same vaccine preparation (homologous prime boost) or using different vaccine preparations (heterologous prime boost) to sequentially stimulate a better immune response.

TETRAMER

A reagent composed of four MHC–peptide complexes linked by biotin and streptavidin, which can be fluorescently labelled and used to track antigen-specific T cells by flow cytometry.

epitopes — sometimes only one. These issues present formidable challenges, but if these problems are properly addressed, the animal models indicate that the vaccine will work.

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Online links

DATABASES

The following terms in this article are linked online to: **LocusLink:** <http://www.ncbi.nlm.nih.gov/LocusLink> CCR5 | CD4 | CD8 | CXCR4 | IFN- α | IFN- γ | IL-2 | IL-12 | MIP-1 α

FURTHER INFORMATION

The International AIDS Vaccine Initiative: <http://www.iavi.org>
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SCIENCE AND SOCIETY – VACCINES

Ethical issues for vaccines and immunization

Jeffrey B. Ulmer and Margaret A. Liu

Vaccination is the only type of medical intervention that has eliminated a disease successfully. However, both in countries with high immunization rates and in countries that are too impoverished to protect their citizens, many dilemmas and controversies surround immunization. This article describes some of the ethical issues involved, and presents some challenges and concepts for the global community.

Vaccines stand out as being among the most efficacious and cost-effective of global medical interventions¹ (BOX 1). Vaccines have saved millions of lives, prevented significant morbidity and suffering, and even eradicated a disease. This last accomplishment, the eradication of smallpox, highlights what can be achieved by vaccination. However, unfortunately, the inequalities in the distribution and use of vaccines are also striking. If vaccines